CASPASE-DEPENDENT CLEAVAGE OF AN IMMEDIATE EARLY HERPES SIMPLEX VIRUS PROTEIN

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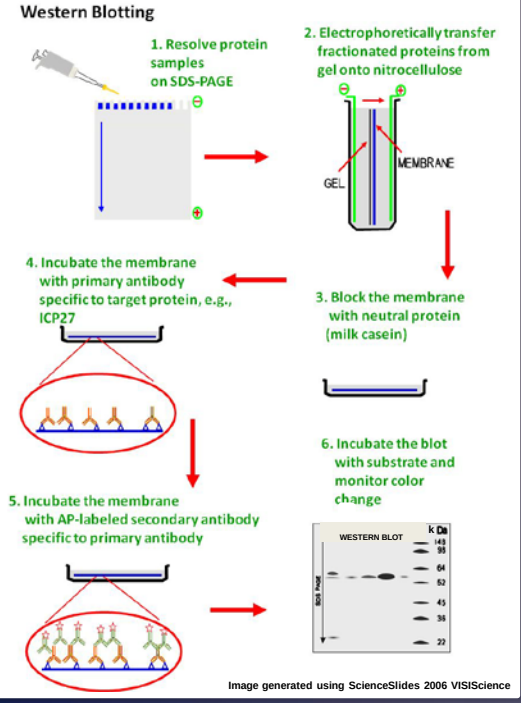
INTRODUCTION

Herpes Simplex Virus1(HSV-1) is a member of the *Herpesviridae* family. All herpes viruses cause life-long infections that shift between latent and lytic stages and have many treatment but no cures. HSV-1 has a double-stranded linear DNA genome, icosahedral capsid, and envelope. HSV-1 can cause infections in humans ranging from encephalitis (brain inflammation) to cold sores.

Previous studies have determined that apoptosis is triggered early during HSV-1 infection. A necessary event in the induction of apoptosis is the activation of a specific set of cellular proteases called caspases. Caspases, cysteine aspartic acid proteases, are proteins that play an important role in cellular protein cleavage during apoptosis. The goal of this study was to determine whether specific viral proteins were cleaved by these caspases. During the course of infection the virus expressed different proteins classified as immediate early, early, and late proteins. These experiments focused on the immediate early proteins, ICP0, ICP4 and ICP27, that regulate expression of the early and late viral genes and are required for virus replication. A late viral protein, VP22, was also analyzed. Human carcinoma Hep-2 cells were used for the infection and western blots were used to detect the viral protein.

Here, we identify the presence of a low molecular weight form of the ICP27 protein in HSV-1 infected cells. The levels of this small form of ICP27 are undetectable when infection are performed in the presence of pan-caspase inhibitors. Together, these results suggest that ICP27 is cleaved in a caspase-dependent manner during infection. Further studies are aimed to confirm this result and elucidate the biological relevance of ICP27 cleavage.

MATERIALS AND METHODS



RESULTS

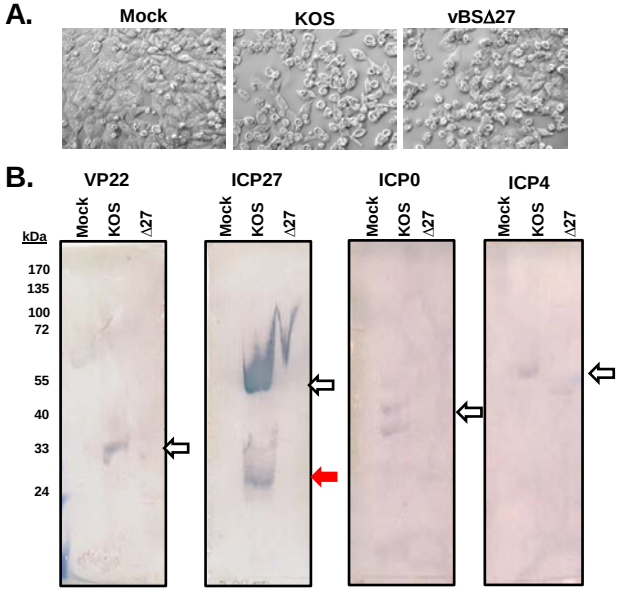


Figure 1: Detection of low molecular weight form of the ICP27. Human carcinoma HEp-2 cells were were infected with wild type HSV-1, KOS 1.1 (KOS) or the apoptotic ICP27-null mutant virus, vBSΔ27 (Δ27) at an MOI of 10. A. Phase contrast photomicrographs were taken at 24 hpi. B. Subsequently, immunoblotting of protein targets for immediate early proteins ICP0, ICP4 and ICP27, along with late viral protein, VP22 was used to pick out any targets for caspase-dependent cleavage. White arrows denote the molecular weight of the full-length forms of each viral protein. The low molecular weight form of ICP27, which migrates at approximately 30kDa, is denoted by a red arrow in the KOS-infected cell lane.

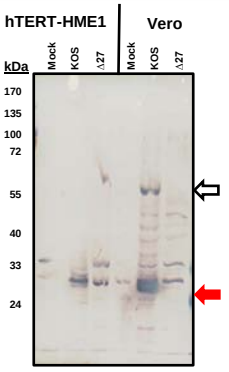
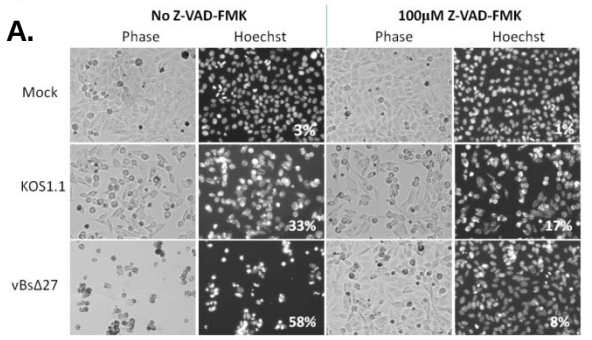


Figure 3: A low molecular weight ICP27 product detected in several cell lines. hTERT-HME1 and Vero cells were infected with wild type HSV-1, KOS 1.1 (KOS) or the apoptotic ICP27-null mutant virus, vBSΔ27 (Δ27) at an MOI of 10. Immunoblotting for ICP27 was completed and showed a fragment (red arrow) in KOS-infected Vero cells that was approximately the same size as the caspase-dependent ICP27 cleavage product observed in Hep-2 cells.



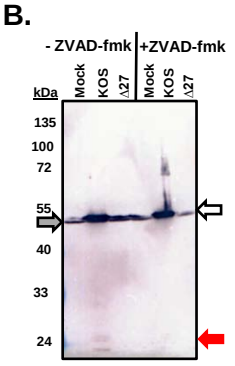


Figure 2: The low molecular weight ICP27 product is inhibited by ZVAD-fmk treatment. Human carcinoma HEp-2 cells were pre-treated for 1 hour with 100μM ZVAD-fmk, a pan-caspase inhibitor, were infected with wild type HSV-1, KOS 1.1 (KOS) or the apoptotic ICP27-null mutant virus, vBSΔ27 (Δ27) at an MOI of 10 in the presence or absence of ZVAD-fmk. The following day, hoechst DNA dye was added to the media of the infected cells and allowed to incubate for 1 hour. A. At 24 hpi, the cells were visualized under phase and UV microscopy for apoptotic morphologies. Images were captured using digital photography. Numbers in the lower right corner of hoechst images represent the percentage of cells displaying apoptotic morphologies. Over 500 cells were counted for each treatment. B. Immunoblotting for ICP27 was done for detection of protein fragments. The full-length form of ICP27 is depicted with a white arrow. A nonspecific band was detected in this immunoblot (gray arrow). The low molecular weight form of ICP27 (red arrow) was present in cells without ZVAD-fmk treatment but undetectable in the ZVAD-fmk treated cells.

SUMMARY

- A low molecular weight ICP27 product was detected in Human carcinoma HEp-2 cells infected with wild type HSV-1.
- The low molecular weight ICP27 product is reduced to nondetectable levels when HEp-2 cells are infected in the presence of a pan-caspase inhibitor. This suggests that the ICP27 fragment is formed through a caspase-dependent pathway.
- A low molecular weight ICP27 product was detected in several cell lines.
- Future studies will be aimed to confirm these result and elucidate the biological relevance of ICP27 cleavage.